labelling occurs and phosphoryl-enzyme is rapidly dephosphorylated. Analyses of hydrolysis products of the acid-denatured labelled protein yielded *O*[^{32}P]-phosphorylserine. Schwartz & Lipmann (1961) presented circumstantial evidence that phosphorylation with orthophosphate at pH 5 occurred at the active site.

The present investigation was designed to determine the rate of dephosphorylation of phosphoryl-phosphatase under conditions when the enzyme was active. The rapid-flow quenching technique (Barman & Gutfreund, 1964) used allows the study of reactions with half-times of the order of 5 msec.

Escherichia coli phosphatase (Worthington Biochemical Corp., Freehold, N.J., U.S.A.) was obtained in the form of a suspension of 2·5 mg. of protein/ml. of 0·65-saturated ammonium sulphate solution. For phosphorylation 0·2 ml. of this enzyme suspension was added to 1·3 ml. of medium: 0·05 M-sodium acetate–acetic acid buffer, pH 5·4, containing magnesium acetate (33 mM) and [^{32}P]-orthophosphate (1·0 mM; 0·025 mC). The rate of combination of [^{32}P]orthophosphate with the enzyme is first-order (0·046 sec.$^{-1}$ at 0°). The [^{32}P]-phosphoryl-enzyme was used within 3 hr. of preparation. Prolonged incubation under these conditions leads to a gradual increase of [^{32}P]orthophosphate bound. For the study of the rate of dephosphorylation the above solution of phosphoryl-enzyme at pH 5·5 was mixed in the rapid-flow system with an equal volume of either the same acetate buffer or 0·1 M-sodium carbonate–bicarbonate containing bovine serum albumin (6 mg./ml.) to give final pH 5·5, 7·3 or 8·4. After specified flow times (see Fig. 1) the reactions were quenched by rapid mixing of the reaction mixture with 7 N-perchloric acid. The kinetic experiments were carried out at 20°. The phosphorylated protein was isolated by centrifuging and separated from contaminating [^{32}P]orthophosphate essentially by the method of Schwartz & Lipmann (1961). The rate of dephosphorylation was calculated from the decrease in the percentage of ^{32}P-labelled enzyme during the time between mixing the phosphoryl-enzyme solution with the appropriate buffer and quenching in perchloric acid. The blank values without enzyme were about 10 % of the maximum bound during several hours at room temperature.

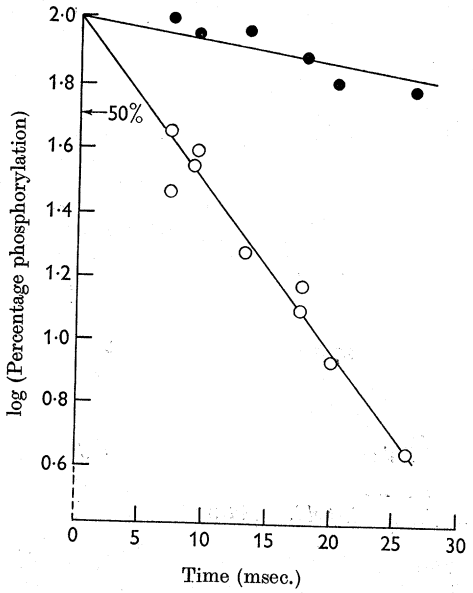

Fig. 1. First-order plot of the dephosphorylation of phosphoryl-phosphatase at pH 7·3 (●) and 8·4 (○). Conditions were as described in the text.

In Fig. 1 the results are shown as first-order plots of the data from experiments carried out at pH 8·4 and 7·3. The half-time of the dephosphorylation at pH 8·4 is 6 msec., which corresponds to a first-order constant 115 sec.$^{-1}$, and at pH 7·3 the half-time is 43 msec., corresponding to a rate constant of 16 sec.$^{-1}$. If the model (Schwartz, 1963):

$$E+ROP \underset{}{\overset{k_1}{\rightleftharpoons}} EROP \overset{k_2}{\rightleftharpoons} EP+ROH$$
$$EP \xrightarrow{k_3} E+P$$

is used to illustrate the proposed mechanism of hydrolysis of the ester (ROP) to ROH and orthophosphate (P) catalysed by the enzyme (E), forming the phosphorylated intermediate EP, one can draw the following conclusions: at pH 8·4 and 20° k_3 is 115 sec.$^{-1}$, and the overall turnover of *p*-nitrophenyl phosphate by the enzyme (k_0) is approx. 30 sec.$^{-1}$. Using the kinetic scheme suggested for such a mechanism by Gutfreund & Sturtevant (1956) one can calculate k_2 as 41 sec.$^{-1}$ from $k_2 = k_0k_3/(k_3-k_0)$. The precise values for the rate constants will need revision when more accurate information is obtained about the turnover per mole of pure enzyme under the conditions of pH, temperature and buffer composition of the present experiment. It should be noted that the first-order constant k_3 may be even larger in the absence of orthophosphate, particularly so at pH 7·3. The qualitative picture shows therefore that the rate constant for the dephosphorylation of phosphoryl-phosphatase is consistent with the proposed mechanism for this enzyme, which involves its transient phosphorylation.

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